Final Technical Report:

(U) Effect of Atmospheric Background Aerosols on Biological Agent Detectors



Prepared for:

Headquarters U.S. Air Force (HQ USAF)/Deputy Director for Counterproliferation (A3SC)

Prepared by:

Jerry G. Jensen

Science Applications International Corporation Hazard Assessment Team 4875 Eisenhower Avenue, Suite 210 Alexandria, VA 22304

This report in its entirety is UNCLASSIFIED

DISTRIBUTION STATEMENT A. Approved for public release; distribution is unlimited.

R	EPORT DOC	UMENTATIO	N PAGE		Form Approved OMB No. 0704-0188		
				wing instructions se	arching existing data sources, gathering and maintaining the		
					collection of information, including suggestions for reducing		
					fferson Davis Highway, Suite 1204, Arlington, VA 22202-		
		R FORM TO THE ABOVE ADDR		or railing to comply v	rith a collection of information if it does not display a currently		
1. REPORT DATE (DD		2. REPORT TYPE		3.	DATES COVERED (From - To)		
01-06-2007	· ·	Technical Report			pril 2004 – January 2006		
4. TITLE AND SUBTIT		Tooliinoai Hopoit			a. CONTRACT NUMBER		
		erosols on Biological	Agent Detectors		A-7014-06-A2003		
Lilect of Attitospile	ilic background Ae	Flosois on Biological	Agent Detectors	'	A-7014-00-A2003		
				51	o. GRANT NUMBER		
				50	:. PROGRAM ELEMENT NUMBER		
C AUTHOR(C)				E.	L DRO JECT NUMBER		
6. AUTHOR(S)				30	I. PROJECT NUMBER		
Jerry G. Jensen							
				50	e. TASK NUMBER		
				0	004		
				51	. WORK UNIT NUMBER		
				31	. WORK ONLY NOWIDER		
7. PERFORMING ORG				-	PERFORMING ORGANIZATION REPORT		
Science Application	ns International Co	orporation (SAIC), Ha	azard Assessment 1	Гeam	NUMBER		
(HAT), 4875 Eisen	hower Avenue. Su	ite 210, Alexandria,	VA 22304				
(,, =	,						
		IAME(S) AND ADDRESS			D. SPONSOR/MONITOR'S ACRONYM(S)		
Headquarters U.S.	Air Force (HQ US	AF)/Deputy Director	for Counterprolifera	ation A	3SC		
(A3SC), HAF/A3S(C, 1480 Air Force F	Pentagon, Washingto	on, DC 20330				
, , , ,	,	<i>y y</i>	,				
				4			
				1	. SPONSOR/MONITOR'S REPORT		
					NUMBER(S)		
12. DISTRIBUTION / A	VAILABILITY STATEN	MENT					
DISTRIBUTION ST	ΓΑΤΕΜΕΝΤ Α. App	proved for public rele	ase; distribution is	unlimited.			
	• •	·	•				
13. SUPPLEMENTARY	NOTES						
13. SUPPLEINIENTART	NOTES						
14. ABSTRACT							
As part of the Kuns	san Focused Effort	(KFE), which was a	project that sought	to develop of	ounter-biological warfare (C-BW)		
strategies and procedures designed to mitigate the impacts of BW attacks on Air Force operations, this technical report							
describes a project that examined both historic and contemporary studies related to background aerosol materials and							
biological weapon (BW) detection systems. The goal was to summarize how current BW detection systems are expected to							
behave in a non-laboratory environment. This report describes past monitoring programs and their key results. The programs							
described were sel	ected because the	eir data results were	directly applicable to	o the backgr	ound problems being analyzed,		
					it are known to adversely affect BW		
detection systems. This technical report illustrates the modeling effort that was undertaken to determine if it was possible to analytically reproduce aerosol concentrations observed by aerosol particle counter devices.							
analytically reprodu	ice aerosol concer	ntrations observed by	y aerosol particle co	ounter device	S.		
15. SUBJECT TERMS							
C-BW CONOPS, BW detection systems, background aerosol impact on biological agent detectors, aerosol particle counter							
		ea, Kunsan Focused			•		
	•	2 2.,	. ,	40 111111	40- NAME OF DECRONORY E DECCON		
16. SECURITY CLASS UNCLASSIFIED	IFICATION OF:		17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON		
			OF ADSTRACT	OF FAGES	Jerry Jensen		
a. REPORT	b. ABSTRACT	c. THIS PAGE	Same as Report	30	19b. TELEPHONE NUMBER (include area		

Unclassified

Unclassified

Unclassified

code)

937-431-4324

(U) Preface

- (U) Between May 2004 and April 2005, the United States Air Force (USAF) conducted an intensive assessment of the biological warfare (BW) threat to operations at Kunsan Air Base (AB), Republic of Korea. This project, known as the Kunsan Focused Effort (KFE), sought to develop counter-biological warfare (C-BW) strategies and procedures designed to mitigate the impacts of BW attacks on Air Force operations and to sustain and recover operations in a broad range of BW environments. The KFE initiative was conducted as a series of visits by a cross-functional team of subject matter experts representing various organizations, e.g., the Air Staff (A3SC), Pacific Air Forces (PACAF), Kunsan AB personnel, Air Force Field Operating Agencies (FOAs), and supporting contractor personnel.
- (U) In support of this effort, a series of seven technical reports were developed to summarize the findings of the KFE analytical efforts. The titles of these technical reports are as follows: KFE Threat Analysis, Residual Hazard Estimation After a Biological Attack, Aerosol and Surface Sampling and Identification Capability Following a Biological Attack, Biological Background Impact on Detector Performance, Analysis of Masking Criteria with Respect to BW Trigger Events, Disease Containment Plan Analysis, and BW Antibiotic Treatment Impact on Casualties and Sortie Generation.
- (U) It is important to note that the seven technical reports were tied to the objectives, guidelines, and timeframes of the KFE program. Therefore, there are some inherent limitations, as well as areas where additional work and analysis is required. These areas have been highlighted in their respective technical report, to include specific recommendations for further analysis.

(U) Abstract

(U) In support of the Kunsan Focused Effort (KFE), this technical report describes a project that examined both historic and contemporary studies related to background aerosol materials and biological weapon (BW) detection systems. The goal of this project was to summarize how current BW detection systems are expected to behave in a non-laboratory environment. This report describes past monitoring programs and their key results. The programs described were selected because their data results were directly applicable to the background problems being analyzed, namely, the nature of background concentrations and fluctuations of aerosol materials that are known to adversely affect BW detection systems. Further, a modeling effort was undertaken to determine if it was possible to analytically reproduce aerosol concentrations observed by aerosol particle counter devices. Input data was obtained from a large number of Met-1 equipped Portal Shield units. Archived data sets were obtained and examined for possible use as input for statistical analyses. These data sets were subject to a variety of time series analyses to determine if descriptive statistics could be obtained and used to produce a statistically similar concentration history. An analysis of these data found that simple particle counters designed to monitor background concentration, such as the Met-1 used in the Portal Shield, are subject to a large number of trigger events. Particle monitor types of triggers all suffer from background concentration fluctuations, which are frequent, natural and man-made, and unpredictable. Each unique type of particle monitor requires a field study to determine how its particular suite of sensors will react to the background materials at different locations. Each new detection technology requires a significant field study effort to determine how the new technology "sees" the environment.

(U) Table of Contents

(U) Preface	1
(U) Abstract	2
(U) List of Figures	4
(U) Acronym List	
(U) Summary	6
(U) Introduction	7
(U) Monitoring Programs and Key Results	8
(U) Particle Counters Used as Trigger Devices	11
(U) Continuous Sample Collection Devices	
(U) Indoor Applications	12
(U) Methods	13
(U) Modeling Background Aerosol Concentrations	13
(U) Conclusions	23
(U) Recommendations	25
(U) Appendix A	26
(U) Appendix B	28

(U) List of Figures

(U)	Figure 1: Osan AB Particles Per Cubic Centimeter (PPCC) Data from April/May	
	1999	13
(U)	Figure 2: Log 10 PPCC at Kunsan AB, 4 – 9 May 2000	14
(U)	Figure 3: Initial Difference Data and PDF of Difference Data	15
(U)	Figure 4: Frequency Distribution Fit to Point to Point Difference (PPCC) Kunsan	
	AB, 4 – 9 May 2000 (Met-1 data)	15
(U)	Figure 5: Reconstruction of the Simulated Signal (PPCC Difference Data) Using a	
	Moving Average Model and Observed Cumulative Distribution Statistics	16
(U)	Figure 6: Reconstructed Time Series from Original and Predicted Difference Data	
	Using a Conditional Probability Technique	17
(U)	Figure 7: Reconstructed Time Series Based on Conditional Probability Approach –	
	No Moving Averages.	17
(U)	Figure 8: Autoregressive to Anything (ARTA) Technique Using Short Input	
	Signal.	18
(U)	Figure 9: Results of Analysis of 353 Background Aerosol Concentration Profiles	
	Using Met-1 Trigger Algorithm	19
(U)	Figure 10: Example of ROC For Several Sets of BAWS Background Data	20
(U)	Figure 11: Example Output From Testbed Using BAWS Sensor Signals Recorded	
	During Various Simulant Releases at Dugway Proving Grounds	21
(U)	Figure A1: Verification of Portal Shield Detection Algorithm	26
(U)	Figure A2: Verification of Portal Shield Detection Algorithm Using a Second Partic	cle
	Size Category.	27

(U) Acronym List

(U) AA Atmospheric Aerosol

(U) AB Air Base

(U) ACPLA Agent Containing Particles Per Liter of Air

(U) ARTA(U) BAWSAuto Regressive to AnythingBiological Agent Warning Sensor

(U) BW Biological Warfare

(U) C-BW Counter-Biological Warfare

(U) C-CBRNE Counter-Chemical, Biological, Radiological,

Nuclear, and High Yield Explosives

(U) CDF Cumulative Distribution Functions

(U) CFU Colony Forming Units
(U) CONOPS Concept of Operations

(U) DARPA Defense Advanced Research Projects Agency

(U) DFU Dry Filter Unit

(U) DNA Deoxyribonucleic Acid

(U) EPA Environmental Protective Agency
(U) FLAPS Fluorescence Aerosol Particle Sizer

(U) HAT Hazard Assessment Team

(U) HHA Hand Held Assay

(U) IBADS Improved Biological Agent Detection System(U) JPBDS Joint Biological Point Detection System

(U) KFE Kunsan Focused Effort

(U) m³ Cubic Meter

(U) MET(U) NMD(U) NMD(U) NRLMeteorological/MeteorologyNumber Median DiameterNaval Research Laboratory

(U) NWCA Nuclear Weapons and Counterproliferation Agency

(U) ORD Operational Requirements Document

(U) PCR
 (U) PDF
 (U) PPCC
 Polymerase Chain Reaction
 Probability Distribution Functions
 Particles Per Cubic Centimeter

(U) PPL Particles Per Liter

(U) Q-PCR Quantitative Polymerase Chain Reaction

(U) ROC Receiver Operating Curves

(U) SAIC Science Applications International Corporation

(U) USAF United States Air Force

(U) UV Ultraviolet

(U) UVAPS Ultraviolet Aerodynamic Particle Sizer

(U) Summary

"A knowledge of the background atmosphere that may be experienced by the detection system is important for establishing the limitations and suitable operating conditions for any sensor."

-- Final Report on the Research Program on BW Detection, Space General, AD480357, 1966.

(U) The goal of this project was to examine the effects of background aerosol materials on the operation of biological agent detection systems currently in use on United States Air Force (USAF) facilities. The effort was initiated as part of the Kunsan Focused Effort (KFE), a project designed to provide a foundation for the compilation of a USAF counter-biological warfare (C-BW) concept of operations (CONOPs). Knowledge of BW detection systems is an important component of the C-BW CONOPs. Statements, such as the one made in the 1966 Space General report, indicate that background aerosol materials have affected and continue to affect the operation of these detection systems. This project sought to examine both historic and current study projects related to background aerosol materials and BW detection systems, and to produce a set of guidelines and conclusions on how current BW detection systems are expected to behave in a non-laboratory environment. The desire was to construct a C-BW CONOPS that would take advantage of knowledge concerning the actual performance of BW detection systems. It is well known that BW detection in general is a difficult problem and that each type of detection system has strengths and weaknesses. Knowing when a BW detection system is likely to work or not work, (specifically as affected by background material), allows other defensive procedures to be implemented, (e.g., increased protection, medical prophylaxis and treatments, etc.), thus providing the most robust defensive capability, while minimizing vulnerability to BW challenges.

(U) Introduction

(U) The Nature of Background Aerosols

- (U) Atmospheric background aerosols have been studied for many years due to their adverse effects on humans and the environment. Fine particles consist of materials that act as aeroallergens, contribution to acid deposition (rain), and reduce visibility. Background aerosols also affect the performance of biological agent detection systems that rely on the capture and identification of aerosol-delivered agent material.
- (U) Atmospheric Aerosols (AAs) are defined as particles with aerodynamic particle sizes of 25 microns or less. AAs are characterized by their source, composition, size, and concentration. Sources of AA include natural flora, vapor condensates (both natural and manmade), and erosion-produced dust. The composition of AAs varies widely and includes pollen, fungal spores, bacteria and bacterial spores, sea spray residues, soot, dust, tire rubber, condensation nuclei, and wind-produced erosion particles. While aerosol particle sizes range from 25 microns to less than 0.1 microns, this report addresses particles in the 10 to 0.5 micron range. These particle sizes are typical of those produced during the dissemination of biological agent materials designed to travel for long downwind distances (up to 100 kilometers). The ability of aerosol particles in the 0.5 to 10 micron range to adhere to deep lung surfaces is one reason that the Environmental Protection Agency (EPA) maintains an aerosol monitoring program. For example, particles larger than 10 microns are considered aerosols, but their larger size and fall velocities limit their aerosol travel distance. Particles smaller than 0.5 microns are ignored due to their limited ability to adhere to deep lung surfaces¹. Moreover, many agents, bacterial agents especially, cannot be delivered in sub-micron particle sizes due to the size of the organism or spore.
- (U) Scientists have known for many years that background atmospheric particles characteristics include: 1) the particles are of the inhalable size (.5-10 microns); 2) the particles can fluoresce (41% of the two to five micron size)²; 3) they contain bacteria and spores³, and 4) the particle concentrations vary by location and time.
- (U) Aerosol concentrations vary widely by location, by time, and by composition. Many aerosol monitoring programs have documented these concentration changes as part of their effort to characterize specific background properties. The aerosol concentration fluctuations over time are important to the biological agent detection problem, as many detector designs are based on detecting the increase or decrease in concentrations that are characteristic of the passing aerosol clouds. A key assumption used in many detection systems is that the concentration change over time from a passing artificially generated aerosol cloud is distinctly different than most natural concentration variations both in time scale, (i.e., time between initial increase and final decrease), and in magnitude of the aerosol concentration.

(U) Background Aerosols and Biological Detection Systems

- (U) Biological aerosol detection systems can be affected by background aerosols in two First, the background aerosols affect systems designed to monitor aerosol concentrations in near real time in order to identify a passing aerosol cloud. Detection algorithms are designed to monitor the ambient aerosol concentration and to detect the increase and decrease concentration pattern of a passing cloud. Simple concentration monitors examine particle counts in one or more size categories. These devices count the number of particles in an air sample. By knowing the volume of the air sample and the number of particles it contains, particle concentration is easily computed. More elaborate systems examine the characteristics of these particles, such as their fluorescence or reflectivity, when they are illuminated by an artificial light source. Such efforts are designed to differentiate between simple non-organic and organic compounds. The idea behind these systems is that organic material (including BW agent) is a fraction of the overall airborne aerosol material, thus a large portion of the background material is Even more elaborate particle concentration monitors are designed to differentiate between different classes of organic compounds. The differentiation between diesel exhaust and other organic materials is one goal of these systems.
- (U) Second, the background aerosol is a concern for the identification process. Many detection systems collect an aerosol sample, concentrate it, and then submit it for identification to some sort of an assay device. The difficulty here is being able to identify an agent particle from the other background material collected in the sample. Various monitoring programs have been performed as a means to identify the range of interfering materials collected and their concentrations so that optimal identification assays can be designed.
- (U) In summary, background aerosol concentrations affect biological detection systems two ways. First, they determine the background concentration and concentration fluctuations monitored by near real time particle concentration monitors. These systems are designed to provide a fast acting trigger signal to either an alarm or a sample collection device. Second, they determine the mix and amount of background material collected and submitted to an identification assay. The identification assay must determine the presence or absence of agent signal from the possible interfering signal originating from the collected background material.

(U) Monitoring Programs and Key Results

(U) This section provides a selected summary of past monitoring programs and their key results. There have been many programs performed worldwide covering a wide range of goals. The programs mentioned here are selected for their data results that are directly applicable the background problems described above, namely, the nature of background concentrations and fluctuations of aerosol materials that are known to adversely affect BW detection systems, particularly in the two ways described above.

(U) M18 Detector Program and Interferents Using Fluorescence

(U) In the late 1970s, the US Army was developing an automated biological detection system that relied on detection of chemiluminescence resulting from a chemical reaction

with luminal and porphyrin⁴. During this time, other research programs were conducted to determine the characteristics of aerosol particles that were commonly found in the atmosphere and their characteristic size. One program reported that almost all pollen particles were relatively large, (i.e., >20 microns) and that fungal spores were almost all around five microns in size⁵. This meant that it was an easy process to exclude pollen from collected aerosol materials. Additionally, this report noted a long list of common materials that fluoresced, including materials of biological origin (both natural and manmade), manmade fibers, and a large number of both organic and inorganic compounds. These data implied that the use of simple fluorescent techniques could not be relied on to filter out non-organic or non-biological materials⁶.

(U) Miscellaneous References and Research Results

- (U) Additional research efforts reported the following results:
 - (U) Forty-one percent of the particles in the two to five micron size range fluoresced⁷.
 - (U) Various types of bacteria were commonly found in/with aerosol particles ^{8,9}.
 - (U) Indoor mineral particles, such as silicate, salt, and others in the three to five micron size range were commonly found in indoor air samples ¹⁰.
 - (U) Time-resolved concentration profiles of bacteria and spores showed that spore concentrations of 11,000 per cubic meter (m^3) occurred and that the concentration curve contained many "bumps" resembling an aerosol cloud passage, as well as many short duration spikes¹¹.
 - (U) A large number of fungi species commonly found in the atmosphere (in Puerto Rico) had concentrations that could reach 250,000 spores/m³, but that the common concentration was a few thousand per m³. The report also contains an estimate that a human inhales between 80,000 and 100,000 spores per day and that there were more airborne fungi during the day than during the night.
 - (U) Regardless of altitude, molds constituted 70 percent of the total airborne microflora; bacteria constituted between 19 and 26 percent; and yeast and actinomycetes filled the remaining percents. The report also noted, "a significant portion of the viable microorganisms in the air were in the particle size range of three to five microns." and that airborne bacteria concentrations increased from 283/m³ to 17,900 /m³ downwind from an activated sludge sewage treatment unit¹³.
 - (U) Many other references indicated the common presence of airborne molds, fungi, and bacteria particles all over the world in similar quantities 14,15,16,17,18,19,20,21
 (U) Of the particle 1 in the common presence of airborne molds, fungi, and bacteria particles all over the world in similar quantities 14,15,16,17,18,19,20,21
 - (U) Of the particle, bacteria, and fungal spore concentrations measured inside homes and daycare centers, bacteria concentrations of up to 8500 colony forming units (cfu)/m^3 were observed. Fungal spore concentrations of up to 5620 cfu/m^3 were reported²². As these data were reported in cfu/m^3, higher airborne concentrations of non-reproducing organisms were certainly present. The report states that there were approximately 50 total airborne bacteria for every culturable bacterium²³.

- (U) One report included a wide list of airborne spore types and concentration statistics for various locations, to include many entries for concentrations above 100,000 spores/m³²⁴.
- (U) (The aerosol) "Background is variable and unpredictable" and "this will impact detector response²⁵."

(U) Improved Biological Agent Detection Systems (IBADS) and Portal Shield Unit Results from Overseas Locations

(U) One report concerned hourly samples collected using the IBADS and Portal Shield units at Osan Air Base (AB) and Kunsan AB in the Republic of Korea, in Kuwait, and Camp Doha, Bahrain. The samples were analyzed using polymerase chain reaction (PCR) and gene sequencing to identify background organisms and to specifically look for the biological agents Bacillus anthracis (B. anthracis), Francisella tularensis (F. tularensis), and Yersinia pestis (Y. pestis). The report did not provide details on the sample collection, but the Portal Shield unit collected for a five-minute duration drawing air at close to one m³/min air flow rate. This is a relatively short duration sample as compared to samples collected by the dry filter unit (DFU) type devices, which collect samples for hours. The results were that zero out of 989 samples from Osan AB were positive for the three agents listed above; one out of 565 samples from Kunsan AB was positive for F. tularensis; one out of 519 samples from Kuwait was positive for F. tularensis; and one out of 516 samples from Camp Doha, Bahrain was positive for both F. tularensis and Y. pestis. These data suggest that about one out of every 500 samples assayed using sensitive identification techniques, (such as deoxyribonucleic acid [DNA] amplification), could be expected to produce a positive result for an endemic biological agent organism. The authors of this report indicated that the amount of agent material in these samples was small, and thus, their results were from endemic organisms rather than deliberately released agents. They did not provide material to indicate how they would determine what level was sufficient to indicate an attack, nor how their techniques could quantify these data. These data suggest the need for an identification technique that is both sensitive and able to quantify the amount of agent material present in a sample ²⁶.

(U) Defense Advanced Research Projects Agency (DARPA) Biological Agent Warning Sensor (BAWS) and Met-1 Results

(U) "A strong mathematical correlation between data from an ultraviolet aerodynamic particle sizer (UVAPS) and simulant concentration was not found." This result indicated that using relatively simple ultraviolet (UV) particle concentration monitoring was not useful as a trigger device because the UV signal did not correlate well with the agent/simulant concentration signal²⁷.

(U) Background Particle Concentrations Around Military Activities

(U) Aerosol measurements were made during a series of trials at Fort Sill, Oklahoma involving truck and tank movements, muzzle blasts, and sub-munition bursts²⁸. These trials were designed to determine the obscuring effects from these activities in both the visual and infrared spectra. The key findings were that the dust particles raised by these activities had number median diameters (NMDs) in the one to four micron range. Additionally, aerosol dosage levels were 0.5 g min/m^3 (peak) for tracked vehicle

operations and about an order of magnitude less for muzzle blasts and sub-munition explosions. Using an assumption of a particle size of three microns, a material density of 2g/cm³, and the observation that the cloud passage time was about two minutes, results in an estimation of aerosol particle concentrations near 1.0 x 10¹⁰ (1e10) particles/m³ for the tracked vehicle activities and 1e9 particles/m³ for the muzzle blast and sub-munition explosions. These are huge concentrations and even if less intensive activities were involved, it is clear that aerosol detection systems will have to deal with a large number of interfering particles in the vicinity of military ground operations.

(U) Particle Counters Used as Trigger Devices

(U) This section provides a brief description of current systems that monitor aerosol particle concentrations in order to provide a relatively fast trigger signal to an aerosol collection system.

(U) Met-1 Used in Portal Shield

(U) The Portal Shield system uses individual Port Shield units each containing a Met-1 real-time particle counter. The Met-1 counts the particles that are present in an air stream in six size categories. The device illuminates air flowing through a tube with a laser and monitors light reflected by aerosol particles contained in the air stream. Properties of the reflected light allow the device to determine the size of the particle being illuminated. The particle counts are reported every nine seconds to a trigger algorithm. The trigger algorithm²⁹ maintains a moving average concentration level to account for natural slow variations in background particle concentrations. The algorithm looks for relatively fast rising concentration values that are characteristics of discrete aerosol clouds, such as would be generated by a remote aerosol generator. If the algorithm detects a possible aerosol cloud, it triggers an aerosol collector/concentrator to collect a sample of the passing aerosol cloud. The Portal Shield units are arranged as an arrayed system in order to minimize the possibility of a small cloud passing the area without detection and to allow multiple units to correlate a cloud. The system algorithm looks at multiple Portal Shield units to see if they trigger in time and space consistent with a passing discrete cloud, based on wind direction and wind speed. If correlated units trigger, then the collected samples from these units are submitted for immunoassay identification, under the assumption that multiple units have detected an aerosol cloud and that the aerosol cloud might be a biological agent cloud. Both the trigger algorithm for individual units and the system correlation algorithm have been tuned, using data collected over a wide variety of locations and for long durations to minimize the number of samples collected and submitted to the identification assay. That is not to say that the algorithm has been tuned to minimize the number of aerosol clouds detected. Experience has shown that the system does a good job of detecting (triggering on) aerosol clouds. Trigger events have been observed to occur in large numbers, especially when vehicle traffic is occurring and during daytime operation.

(U) Biological Agent Warning System (BAWS)

(U) BAWS is an advanced particle monitor developed by Lincoln Laboratories to supply the particle monitoring function for the Joint Biological Point Detection System (JBPDS) and to replace or augment the Met-1 device in the currently fielded Portal Shield systems.

Like the Met-1, BAWS illuminates the air flowing in a tube with a laser (UV laser in this case), and monitors three spectral signals returned by entrained particles. These signals indicate the relative concentration of the aerosol, (but not the actual particle count), and allow the illuminated material concentration estimates to be placed in material category bins. The idea behind the device is that category bin(s) associated with background interferents are different from the bins that contain agent materials, (i.e. they look different in the three different spectral bands). A large monitoring program was performed to determine the spectral returns of normal background materials and their bin characteristics. Likewise, laboratory tests were undertaken to determine the spectral signals returned by agent materials to establish their bin characteristics. The BAWS unit contains a trigger algorithm based on these data and the moving average of the background concentration. The goal of the BAWS is to eliminate many of the trigger events produced by the more simple Met-1 that are caused by natural or manmade background aerosol clouds that do not contain agent (or possible agent). As with the Met-1/Portal Shield program, a large BAWS monitoring program was undertaken to characterize the BAWS background signals and to tune the trigger algorithm.

(U) Continuous Sample Collection Devices

(U) Rather than rely on a trigger device to turn on an aerosol collector and then initiate a possible identification process, a second type of point biological agent collector, called a continuous sample collector, is in common use. These devices draw a continuous air stream through an inlet (designed to separate out large particles), concentrate the particles of the desired size range, and collect these particles either on a filter medium or in a liquid. The collected sample is transported to a laboratory for sample processing and analysis. Because these devices are continuously collecting aerosol material, they are not susceptible to missing an agent cloud due to the failure of a trigger device. These devices typically collect aerosol samples with collection durations ranging from one to 24 hours. The shorter sample intervals result in the ability to detect a possible attack quicker than the longer sampling intervals, assuming the individual samples are processed promptly. Disadvantages to this approach are as follows: 1) each sample must be processed; 2) there is a significant time delay between sample collection and sample processing results; 3) sample material remains on/in the collection media for a long period of time, which may adversely affect the sample processing step, (e.g., vegetative bacterial cells cannot be cultured after drying out on filter media); and 4) large amounts of background material are included in the sample.

(U) Indoor Applications

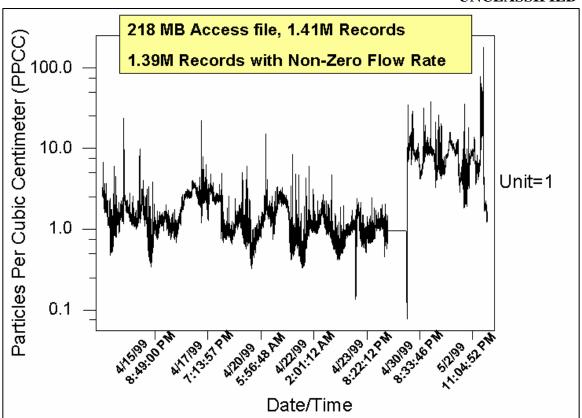
(U) DARPA undertook a program to examine the ability of particle counters and BAWS to ignore background aerosol fluctuations that normally occur in a variety of occupied buildings. Their results showed that particle counters could only operate at a reasonable false alarm rate if their sensitivity was adjusted to 2000 - 3000 particles per liter (PPL) background concentrations. The BAWS performed better requiring a sensitivity of 300 PPL. The trigger events observed at these sensitivities were often, but not always associated with cleaning activities.

(U) Methods

(U) Modeling Background Aerosol Concentrations

(U) A modeling effort was undertaken to determine if it was possible to analytically reproduce aerosol concentrations observed by aerosol particle counter devices. Input data was obtained from a large number of Met-1 equipped Portal Shield units. Of note, Met-1 measurements and other Portal Shield data are routinely archived, which resulted in a plethora of data. A number of these archived data sets were obtained and examined for possible use as input for statistical analyses. Examination of the raw data indicated that manual processing was required due to time gaps and other obvious data anomalies (reference Figure 1).

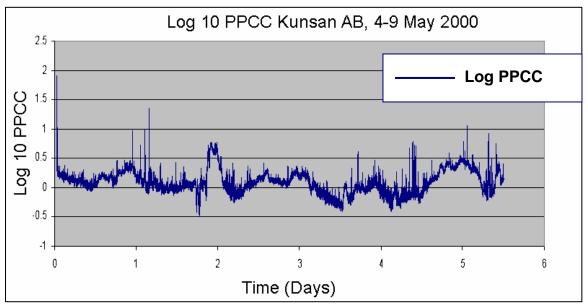
UNCLASSIFIED



(U) Figure 1: Osan AB Particles Per Cubic Centimeter (PPCC) Data from April/May 1999. This dataset contains obvious discontinuities. Three hundred and fifty three datasets were edited so only continuous sampling data were analyzed.

(U) Of the data sets obtained, only 353 passed the acceptance criteria of being continuous in time and having a duration period of at least six hours. Figure 2 is an example of one of these data sets.

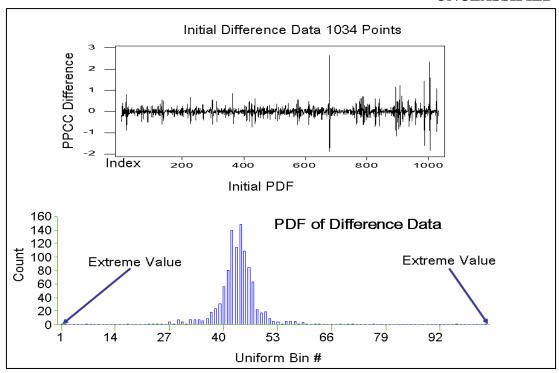
UNCLASSIFIED



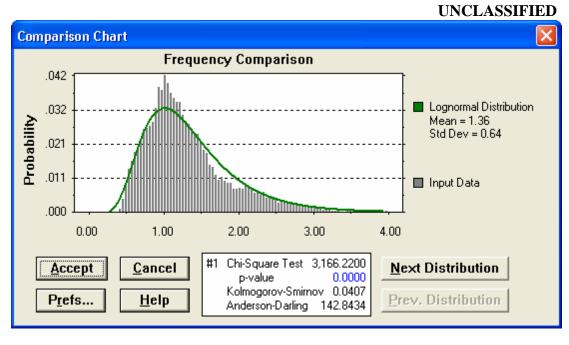
(U) Figure 2: Log 10 PPCC at Kunsan AB, 4 – 9 May 2000. This is an example of an accepted dataset containing no time gaps.

(U) These data sets were subject to a variety of time series analyses to determine if descriptive statistics could be obtained and used to produce a statistically similar concentration history. One approach was to compute the point-to-point difference in particles per cubic centimeter (PPCC), the data value reported by the Met-1, for a particular size channel. The top curve of Figure 3 shows an example computed from the Figure 2 data. The difference data was then fitted with either a standard probability distribution functions (PDF) using a statistical analysis program (Figure 4), or an empirical PDF (bottom of Figure 3) that was computed directly from the data.

UNCLASSIFIED



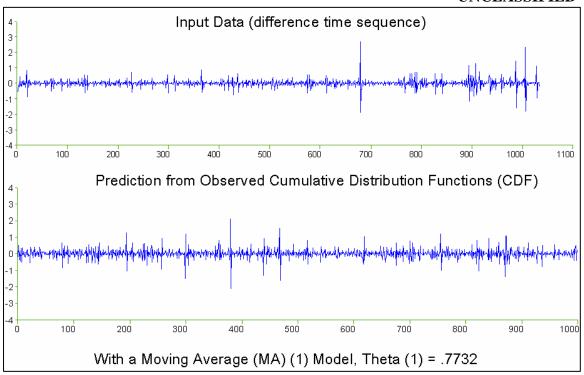
(U) Figure 3: Initial Difference Data and PDF of Difference Data. This chart is a point-to-point difference in PPCC computed from the Figure 2 data (Kunsan AB, 4-9 May 2000). The extreme value left is -1.9 and the extreme value right is 2.7. There are 100 uniformly spaced bins and the mean of distribution is 0.



(U) Figure 4: Frequency Distribution Fit to Point-to-Point Difference (PPCC) Kunsan AB, 4 – 9 May 2000 (Met-1 data). This chart is the difference data fitted with an empirical PDF computed directly from the data.

(U) A simulated difference series is then produced by random sampling from the PDF or by using a moving average method combined with random sampling from the PDF. The bottom of Figure 5 shows the results of such a process using statistical data computed from the curve shown in the top portion of the figure. Although this simulation of the original difference data looks similar, the process of reconstruction of the simulated signal (from the difference data) produces results that are obviously different as shown in Figure 6. The downward pointed spikes in the simulated signal are not present in the original signal and suggest that some sort of time correlation is needed with the difference data to ensure that upward spikes are followed at some time by a downward spike of similar magnitude. Various types of moving average (MA) and conditional probability schemes were attempted to rectify this problem.

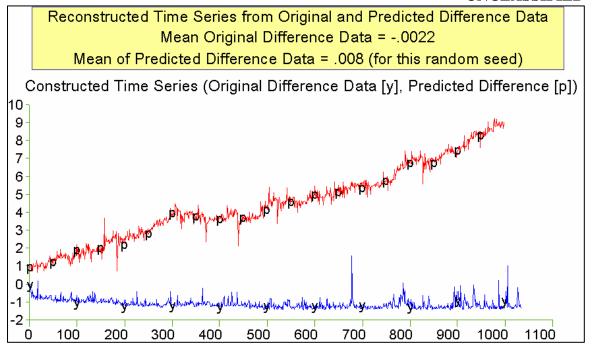
UNCLASSIFIED



(U) Figure 5: Reconstruction of the Simulated Signal (PPCC Difference Data) Using a Moving Average Model and Observed Cumulative Distribution Statistics.

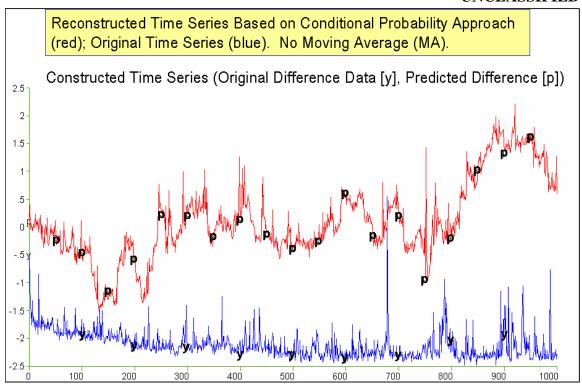
(U) The moving average techniques were able to reproduce the difference data well (reference Figure 5), however, they failed when concentration features, (such as an increase in concentration caused by a passing aerosol cloud), were present having time scales different from the moving average scale. The conditional probability techniques failed by not having enough memory, which allowed the simulated signal to "wander" (reference Figures 6 and 7).

UNCLASSIFIED



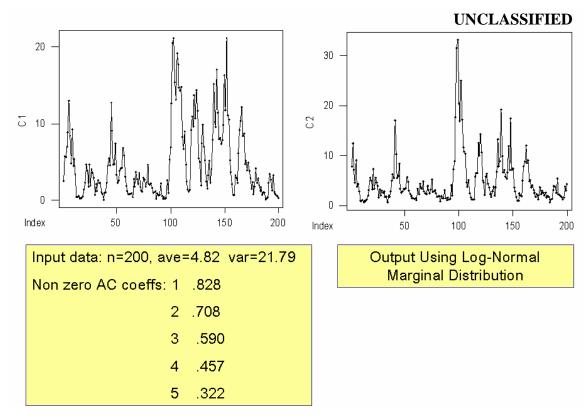
(U) Figure 6: Reconstructed Time Series from Original and Predicted Difference Data Using a Conditional Probability Technique. Y scales offset for clarity.

UNCLASSIFIED



(U) Figure 7: Reconstructed Time Series Based on Conditional Probability Approach – No Moving Averages. Y scales offset for clarity.

(U) The best results were obtained using an auto-regression technique³⁰. This process. called Autoregressive to Anything (ARTA), combines auto-regression statistics for the time series data with a probability distribution to generate a simulated time series that has the correct auto-correlation and cumulative statistics. Figure 8 shows the results of this technique on a short input signal. This technique can generate realistic simulated background concentration time series data, but these data contain no more "information" than the initial data sets used to compute the auto-correlation coefficients and the range of values contained in the PDF. The ARTA process contains a wide range of available PDFs, so the realism of the simulated time series depends on how well the chosen PDF matches the characteristics of the real background data. The conclusion for this modeling effort was that there are time series simulation techniques that can generate time series data that may be useful for some simulation and modeling applications. However, these generated data sets cannot contain any more information about the background concentration than the real concentration time series data used to generate the simulation generation statistics. Furthermore, this modeling effort did not attempt to replicate any of the spatial correlation. This additional level of complexity would be necessary for simulating the operation of detection arrays that utilize multi-sensor spatial and temporal correlations associated with cloud traversal across detection arrays utilizing complex multi-detector algorithms.

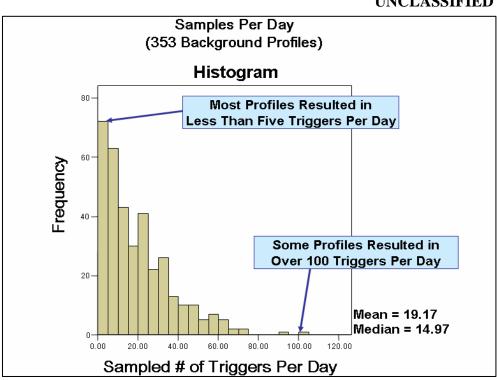


(U) Figure 8: Autoregressive to Anything (ARTA) Technique Using Short Input Signal.

(U) Met-1 Performance

(U) Data used to generate artificial background aerosol profiles were also used to analyze the Met-1 performance with respect to false triggers. The 353 background profiles containing over 2000 hours of Met-1 samples were submitted to the Portal Shield trigger algorithm to determine the number of trigger events expected over a period of time. Details of this Portal Shield/Met-1 trigger analysis are in Appendix A. The results of this analysis (reference Figure 9) indicated that the mean number of samples per day per Met-1 is about 19 (median of 15). There were some instances where the background concentration fluctuations were so common that a Met-1 would be expected to trigger over 100 times per day (essentially continuously). Because of the design of the trigger algorithm, each trigger event is caused by a concentration time profile that resembles the passage of an aerosol cloud. Because the Met-1 device is a mature sampling device, it is likely that these trigger events were caused by aerosol clouds (and not by spurious Met-1 operating anomalies). If these trigger events were real aerosol clouds, then there is every reason to assume that these clouds would constitute a system trigger for the Portal Shield array operating in smart mode. In other words, these clouds, assuming they were large enough to cover multiple Portal Shield units, would be correlated with wind direction and wind speed so that the Portal Shield system would indicate that the cloud was a possible biological aerosol cloud and cause the samples collected by the triggered units to be submitted for immunoassay identification. The conclusion is that the Portal Shield system operating in smart mode would be expected to trigger, (i.e., initiate immunoassay identification at the triggered units) close to 19 times per day (average) based on the collected data analyzed here.

UNCLASSIFIED

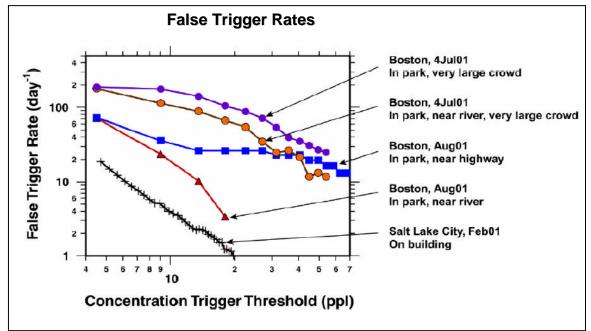


(U) Figure 9: Results of Analysis of 353 Background Aerosol Concentration Profiles Using Met-1 Trigger Algorithm.

(U) BAWS Performance

(U) As with the Met-1 trigger unit of the Portal Shield, the BAWS trigger unit was subject to widespread field use to determine its performance. The BAWS developers report BAWS performance (and biological agent detection systems in general) as receiver operating curves (ROC). Figure 10 shows an example of ROC for a few sets of BAWS background data. These curves show the trade-off between detector sensitivity and false positive rate³¹.

UNCLASSIFIED



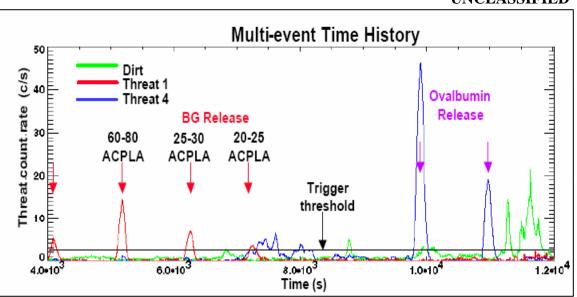
(U) Figure 10: Example of ROC For Several Sets of BAWS Background Data³².

(U) The BAWS developers have a software system called Testbed that allows the developers to take recorded BAWS raw sensor signals and vary the detection algorithm parameters for optimal results. Figure 11 shows an example output from Testbed using BAWS sensor signals recorded during various simulant releases at Dugway Proving Grounds. The BAWS developers can adjust how the raw sensor data is compiled into threat counts data, which represent the intensity of possible agent aerosol concentrations. Testbed also allows the threshold trigger level to be adjusted so that true triggers, (i.e., when the threat count for a known release is above the threshold level) are maximized and false triggers are minimized.

(U) The adjustment process was done in two parts. The threat count parameters were adjusted using data from known simulant releases and agent signatures obtained in aerosol chambers. The simulant release test data included the signal from the background aerosol. Whereas, the agent releases occurred in controlled aerosol chambers and thus, the recorded data included no outside background aerosol effects. During these tests, other referee samplers determined the agent or simulant cloud's aerosol concentration, which allowed the threat count data to be converted to agent/simulant concentration, as measured in agent-containing particles per liter of air (ACPLA). The threshold trigger level was initially adjusted using recorded data from various BAWS sampling locations.

Long term sampling at various locations allowed the background signals to be recorded and characterized so that the number of false triggers per day versus threshold trigger level can be determined. The BAWS developers then combined the background trigger level data with the agent/simulant data to determine the ROC as shown in Figure 10. These data showed that the sensor's performance was location specific and that local optimization required data to be collected over a long period of time in order to perform local tuning.

UNCLASSIFIED



(U) Figure 11: Example Output From Testbed Using BAWS Sensor Signals Recorded During Various Simulant Releases at Dugway Proving Grounds. Raw spectral sensor values were processed into threat counts. The trigger threshold was then adjusted to maximize threat triggers and minimize false triggers from dirt and other unknown materials³³.

(U) As part of this project, the BAWS Testbed software system was obtained and used to analyze a few recorded BAWS data sets. However, the complexity of this system precluded a large-scale analysis of various BAWS location data sets, as was done with the Portal Shield Met-1 data.

(U) Since these data were presented, the BAWS developers have continued to improve the BAWS trigger algorithm. Data presented at the DARPA Special Projects Office Sensor Testing Workshop on 9 December 2004³⁴ indicated that the BAWS has a false trigger rate between one and 10 per day at a threshold level of 100 ACPLA and between 10 to 100 false triggers per day for a threshold level of 25 ACPLA³⁵. This ROC data is a result of long term (duration) field-testing in Boston, Missouri, Atlanta, Cambridge, and Kuwait, among other locations. These data showed a wide degree of variation from location to location, so these false trigger rates were just estimates for an unknown location. Other data collected from BAWS indicated that the device had problems associated with diesel exhaust and particles associated with subway train operations. Further, the more sophisticated optics used in this device required more frequent cleaning (than a Met-1 type device) in order for the device to operate at nominal levels. Given the time over which the BAWS has been developed, and the fact that improved versions of

BAWS have been proposed, this suggests that these data represent the performance limits of current technology. How well this device can be expected to perform at any untested location cannot be predicted.

3 4 5

6

7

8

9 10

11 12

13

14

15 16

17

18 19

20

21

22

23

24

25

26

27

28

29

30

31

1

2

(U) Continuous Sample Collection Device Performance

(U) Testing performed by DARPA (reference the DARPA Sensor Testing Workshop) showed that vegetative bacterial cells cannot be cultured after drying out on filter media, and that filter media residue affects PCR assays. Background material was shown to reduce the sensitivity of some assays, (e.g., HHAs) by a factor of ten³⁶. The effect of background material on PCR assays has not been determined. PCR analysis of material collected on filter media resulted in a false positive rate of about 1% 37. This false positive rate can be reduced (amount unspecified) by applying an independent secondary assay on other genetic loci within the organism. Multiple officials associated with the Biowatch¹ program have stated that millions of samples have been processed without a false positive ³⁸. This statement conflicts with the above false positive data and with the Naval Research Laboratory (NRL) report that found endemic F. tularensis and Y. pestis in one out of every 500 samples³⁹. A Biowatch official stated that endemic agent material is detected using quantitative PCR (Q-PCR). This technique relies on counting the number of amplification cycles necessary to increase the target signal to a detectable level. Samples with small amounts of initial segment material, (i.e., assumed to be small amounts of endemic background material) require a larger number of amplification cycles than samples with large amounts of initial segment material, which are assumed to be collected from an intentional biological release. The number of amplification cycles required for detection of the sample can be compared to the number of cycles required for detection of a positive control to determine relative initial sample concentration. This is a controversial approach to filtering out endemic agents since the sample may contain PCR inhibiting material. Although Q-PCR is routinely used to quantify sample material in laboratories, this application relies on well-characterized samples. The reliability of O-PCR techniques on samples containing unknown background materials has not been established. These data suggest that the relationship between background material and its effect on detection/identification for continuous samplers is not well known at this time.

32 33

34

_

¹ Biowatch is an environmental monitoring system developed by the Department of Homeland Security that monitors air samples on a frequent basis in major urban cities across the U.S.

(U) Conclusions

(U) Particle Monitors/Triggers

- (U) Simple particle counters designed to monitor the background concentration of particles in various size categories, such as the Met-1 used in the Portal Shield, are subject to a large number of trigger events. These trigger events are caused by passing aerosol clouds that occur naturally and from human activities. Experience with simple particle counters has shown that the number of trigger events is generally too high, especially when used around heavy human activities when the detection level is set to a useful (adequate sensitivity) level.
- (U) Fluorescent type particle counters, e.g., Fluorescence Aerosol Particle Sizer (FLAPS), are more sophisticated, in that they count particles that fluoresce under the idea that most background materials are inorganic and thus non-fluorescing. Multiple studies indicated that much of the background material fluoresces and fluorescent particle concentration does not correlate well with agent/simulant concentration. A simple FLAPS device offered little improvement over simple particle counters.
- (U) Sophisticated multi-spectral monitors look at reflected light from illuminated particles at multiple wavelengths under the idea that various types of particles and organisms have different spectral signatures. The BAWS is an example of this type of instrument. Field results indicated that these devices are still plagued by background materials.
- (U) Particle monitor triggers all suffer from background concentration fluctuations, which are frequent, natural and man-made, and unpredictable. Each unique type of particle monitor requires a field study to determine how its particular suite of sensors will react to the background materials at different locations. In order to operate these devices at reasonable sampling rates, their sensitivities are adjusted to the point where they are able to trigger on only the more intense concentration events. Their operational sensitivities are not adequate to satisfy operational requirements document (ORD) requirements. It is important to note that the term "trigger event" is used rather than the term "false positive," as these devices initiate a sample collection and possible identification sequence. These devices are designed to detect specific aerosol concentration signatures that might indicate the presence of a BW agent cloud. These concentration signatures occur all too frequently. These are not false alarms.
- (U) The effects of background aerosols on new detection equipment technologies cannot be predicted. Each new detection technology requires a significant field study effort to determine how the new technology "sees" the environment. Experience has shown that the environment is very different for different locations and sampling times. This means that such field study activities must occur over many locations and relatively long periods of time. In cases where the detectors are fielded as arrays, and include multi-detector algorithms, field testing of a multi-detector subset is the only way to determine the system's background response characteristics. Existing data sets collected from detectors/samples of a different design, cannot be used to estimate the expected

performance characteristics of a different type of system. This makes it difficult/expensive to perform the field testing necessary to gain confidence in the level of increased performance for any new detection system.

(U) Triggered Detection Systems

(U) Detection systems such as Portal Shield and JBPDS consist of arrays of detection units that require correlated triggers (operating in Smart Mode) before sample identification procedures are initiated. Positive agent identification of collected samples must be confirmed by a second identification assay before the possible presence of agent is declared. In such a case, a sample is subject to yet another independent identification assay before an alarm signal is issued. Because of these requirements for multiple types of identification assays, current BW detection systems have very low system level false alarm rates. Background aerosol materials contribute to increased rates of individual sensor sampling activities. Increased rates of sampling, due to background aerosols with resulting negative identification results, is often, and incorrectly referred to as false sampling or false triggering. The trigger devices are performing as they are designed. The background aerosol material increases the number of trigger/sample events, which usually leads to an increase consumption rate of identification reagents. If the sampling rate and reagent consumption rate is deemed too high, then either the system is turned off or the trigger thresholds are raised. In either case, the detection components and total system are operating at a reduced sensitivity. The system user is provided no guidance as to the level of performance of such a degraded system.

(U) Continuously Collecting Systems

(U) Little technical data is available relating the effects of background aerosol levels on continuously collecting detection systems. The Air Force does not use these systems routinely. Such systems have fixed reagent consumption rates and require transportation and laboratory support for sample transport and processing. The Biowatch program utilizes this type of detection device, but detailed technical results were not available for analysis during this effort. Results from this program and the NRL effort suggest that background material does not contribute to false positive results. Results of the DARPA effort and the fact that Biowatch does not react to endemic agent material that must be in their samples suggests that current processes are not very sensitive, although good data to support this conclusion is not available.

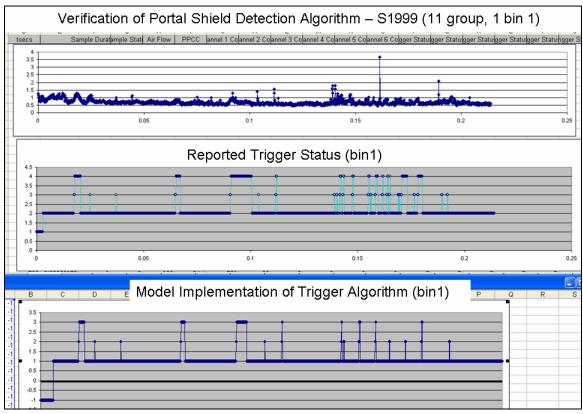
(U) Recommendations

- (U) After a BAWS has been in the field for some time, it is possible to construct a local ROC so that false trigger rates could be balanced against the threshold trigger level and its relation to the current threat condition. When BAWS is used with systems using identification technologies of limited sensitivity, such as the JBPDS, it is possible that the limiting detection factor is the identification stage, and not the background induced false trigger rate. An effort should be made to investigate the possibility of generating operational guidance on the relationship between BAWS trigger threshold level, false trigger rate, and threat based on local ROC data.
- (U) As more sensitive identification technologies, such as PCR come on-line, it will become more important to be able to distinguish endemic agent background material from deliberate agent releases (attacks). An effort should be made to collect the raw assay data from the Biowatch program and examine it to determine frequency and characteristics of sampled endemic agent material. These data will be useful for the determination of the ROC data for both triggered and continuous sampler systems using PCR identification.

(U) Appendix A (U) Modeling Portal Shield/Met-1 Trigger Algorithm to Determine the Number of Trigger Events Resulting from Various Concentration Time Series

(U) The Portal Shield/Met-1 trigger algorithm was obtained from (Sentel Corporation⁴⁰) and implemented as a computer code to generate Met-1 trigger events in response to an input concentration time series, as it would be obtained directly from the Met-1 device. The algorithm is complex and an effort was made to validate its implementation by comparing the newly coded algorithm results to records of actual Portal Shield trigger responses. The data used to analyze background aerosol concentrations was reported by Met-1 devices within Portal Shield units at various times and locations. These archived datasets also contained data indicating the state of the real trigger algorithm. Figure A1 shows the results of one of these validation tests.

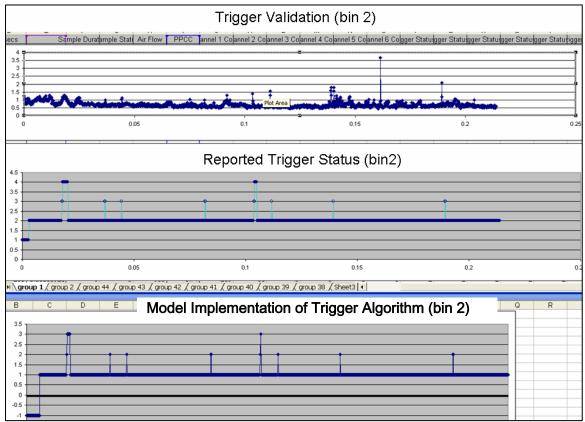
UNCLASSIFIED



(U) Figure A1: Verification of Portal Shield Detection Algorithm. The top curve is point-to-point difference (PPCC). The middle curve shows the reported Portal Shield trigger state from bin 1 particle concentration data. The bottom curve is the computed trigger state determined by the implementation of the Portal Shield trigger algorithm.

(U) The input concentration signal (in particles per cubic centimeter from bin1) is shown as the top curve. Bin 1 is one of the Met-1 size channels. The reported trigger state is shown as the middle curve. Trigger states are as follows: 1 = initializing; 2 = operating (green); 3 = yellow (possible trigger signal detected); and 4 = red (trigger signal). The results of the new code are shown as the bottom curve. The reported trigger states in the bottom curve are the same as middle curve, but are offset by one unit, (i.e., 3 = red rather than 4 = red). Examination of these results shows that the trigger algorithm implementation is very close to the performance of the actual trigger algorithm. There are some differences in de-trigger timing (how the algorithm resets) and in the presence of very noisy signals. Figure A2 shows the results of the trigger algorithm validation for another particle size bin. Again, good results are obtained. Qualitative comparisons were made for three more sample profiles with similar levels of accuracy. Based on theses comparisons, the code was deemed sufficient for use in processing the remaining Met-1 type concentration profiles for use in computing the number of trigger events that can be expected from the wide range of available background aerosol data sets.

UNCLASSIFIED



(U) Figure A2: Verification of Portal Shield Detection Algorithm Using a Second Particle Size Category. The top curve is point-to-point difference (PPCC). The middle curve shows the reported Portal Shield trigger state from bin 1 particle concentration data. The bottom curve is the computed trigger state determined by the implementation of the Portal Shield trigger algorithm.

(U) Appendix B

(U) References

⁴ Berry, P., and Keane, W., US Army Chemical Systems Laboratory, *The Biological Detector and Warning System, XM19/XM2*, ADA090364, Aberdeen Proving Ground, MD, June 1980.

⁵ Putscher, R. et al., Characterization of Air Particles Giving False Responses with Biological Detectors, Walter C. McCrone Associates, Inc., ADA015519, prepared for Edgewood Arsenal, July 1975.

⁶ Ibid.

⁷ Task II of Research Program on BW Detection, *Final Report on the Research Program on BW Detection*, Space General, AD480357, SGC 382R-8, Volume I, Technical Discussion, 1966.

⁸ Prepared for National Science Foundation, International Biological Program, *Aerobiology and its Modern Applications: A Discipline of Investigations of Aerial Transport of Biological Materials Important to Human Health and Welfare*, Distributed y National Technical Information Service, U.S. Department of Commerce, PB-225-535, July 1973.

⁹ U.S. Army Chemical Corps, Dugway Proving Ground, *The Indigenous Bacterial and Mycotic Flora of the Air and Its Influence on the Detection of BW Aerosols*, Technical Report DPGR 279, AD 257 336, February 1961.

¹⁰ Kamens, R., Lee, C., Wiener, R., and Leith, D., *A Study to Characterize Indoor Particles in Three Non-Smoking Homes*, Department of Environmental Sciences and Engineering at the University of North Carolina, Chapel Hill, U.S. Environmental Protection Agency, Atmospheric Research and Exposure Assessment Laboratory, and Department of Civil Engineering, National Central University, Taiwan, PB91-242982 and EPA/600/J-91/206, final form dated 3 August 1990.

¹¹ Tilley, R., Ho, J., and Eamus, D., *Background Bioaerosols and Aerosols at Two Sites in Northern Australia: Preliminary Measurements*, ADB272376 and DTSO-TR-1203, Department of Defence, Defence Science and Technology Administration, August 2001.

¹² Department of the Army, Fort Detrick, *Atmospheric Fungi*, AD838549, Translation No: 1336, April 1965.

¹³ Finkelstein, H., for Litton Systems, Inc., Environmental Systems Division, *Air Pollution Aspects of Biological Aerosols (Microorganisms)*, prepared for National Air Pollution Control Administration, Consumer Protection & Environmental Health Services, Department of Health, Education, and Welfare, September 1969.

¹⁴ Al-Frayh, A., Hasnain, S., Wilson, J., and Harfi, H., *Fungal Allergens in the Atmosphere of Riyadh: A Preliminary Communication*, Annals of Saudi Medicine, Volume 8, Number 4, 1988.

¹⁵ Consentino, S., Pisano, P., Fadda, M., and Palmas, F., *Pollen and Mold Allergy: Aerobiologic Survey in the Atmosphere of Cagliari, Italy (1986-1988)*, Annals of Allergy, Volume 65, November 1990.

¹⁶ Vittal, P., and Krishnamoorthi, K., A Census of Airborn Mold Spores in the Atmosphere of the City of Madras, India, Annals of Allergy, Volume 60, February 1988.

¹⁷ Anderson, J., Allergenic Airborne Pollen and Spores in Anchorage, Alaska, Annals of Allergy.

¹⁸ D'Amato, G., Stanziola, A., Cocco, G., and Melillo, G., *Mold Allergy: A Three-Year Investigation* (1980-1982) of the Airborne Fungal Spores in Naples, Italy, Annals of Allegy, Volume 52, May 1984.

¹⁹ Halwagy, M., *Seasonal Airspora at Three Sites in Kuwait*, Mycological Research, 93 (2): 208-213, 1989, printed in Great Britain.

²⁰ Ebner, M. and Haselwandter, K., *Seasonal Fluctuations of Airborne Fungal Allergens*, Mycological Research, 92 (2): 170-176, 1989, printed in Great Britain.

²¹ Hallin, P., Linfors, G., and Sandstrom, G., *Investigation of Variation in the Concentration of Bacteria at Outdoor Testing with the Use of a Detector for Aerosols of Bacteria*, National Defence Research Institute, Department 4, FOA report C 40201-B2, September 1984.

²² Pellikka, M., Pitkanen, E., Nevalainen, A., Jantunen, M., and Kalliokoski, P., *Partikkeli – Bakteeri – Ja Sieni –Itiopitoisuudet Ilmalammitteisissa Pientaloissa, Paivakodeissa Ja Muutamissa Valituskohteissa,* LVI-Tekniikan Laboratorio, TKK-KO/LVI—Raportti C-25, ISBN 951-753-845-6, DE87 752387.

¹ Kennedy, G., and Valentine, R., *Principles and Methods of Toxicology*, Chapter 22, 1994.

² Final Report on the Research Program on BW Detection, Space General, AD480357, 1966.

³ Ibid

²³ Variations in the Quality and Quantity of Natural Atmospheric Bacteria (Background) Populations, presented at the Fourth Joint Workshop on Standoff Detection for Chemical and Biological Defense, Oct. 1998.

²⁴ Rose, W., *Naturally Occurring Atmospheric Bioflora*, Technical Analysis and Information Office, U.S. Army Dugway Proving Ground, ADB111630, DPG/TA-87-08, TECOM Project Number 8-CO-260-FAT-001, March 1987.

²⁵ Briefing by Birenzvige, A., Lowrey, A., and Kierzewski, M., "Sample Volume Model for Enhancing Biological Detection," ERDEC, NRL, and Optimetrics.

²⁶ Francesconi, S., Worth, L., Churilla, A. (CDR), Campbell, J. (CAPT), *Molecular Biological Characterization of Air Samples: A Survey of Four Strategically Important Regions*, Naval Research Laboratory, NRL/MR/6110-03-8661, #20030312 219, January 31, 2003.

²⁷ Defense Advanced Research Projects Agency (DARPA), Special Projects Office, Sensor Testing Workshop, Arlington, VA, 9 December 2004.

²⁸ U.S. Army, Dugway Proving Ground, *Dust/Debris Field Test (Add On)*, U.S. Army Test and Evaluation Command, ADA066377 and ADA069154, DPG-FR-68-313, Volume I, TECOM Project No: 7-CO-RD8-DP1-005.

²⁹ Birmingham, W., *Description Portal Shield Trigger Algorithm*, PSTRIG.DOC, Sentel Corporation, February 1998.

³⁰ Cario, M. and Nelson, B., *Numerical Methods for Fitting and Simulating Autoregressive-to-Anything Processes*, Delphi Packard Electric Systems and Department of Industrial Engineering and Management Sciences, February 1997.

³¹ Carrano, J (LTC), Jeys, T., et al., Defense Advanced Research Projects Agency, DARPA Chemical and Biological Sensor Standards Study.

³² Massachusetts Institute of Technology (MIT) Laboratory, JBPDS 2001 – 15, THJ, November 30, 2001.

³³ Data presented by MIT Lincoln Labs Biological Defense Program Overview, December 2001.

³⁴ Defense Advanced Research Projects Agency (DARPA), Special Projects Office, Sensor Testing Workshop, Arlington, VA, 9 December 2004.

³⁵ BAWS Objective Level-Ref. Sensor Systems for Bio Agent Attacks: *Protecting Buildings and Military Bases*, National Research Council, 2004.

³⁶ Defense Advanced Research Projects Agency (DARPA), Special Projects Office, Sensor Testing Workshop, Arlington, VA, 9 December 2004.

³⁷ Abarbanel, H., Block, S., Drell, S., Dyson, F., Henderson, R., Koonin, S., Lewis, N., Schwitters, R., Weinberger, P., and Williams, E., *Biodetection Architectures*, JSR-02-330, The Mitre Corporation, February 2003.

³⁸ Hsu, S., Anthrax Alarm Uncovers Response Flaws, Washington Post, March 17, 2005.

³⁹ Francesconi, S., Worth, L., Churilla, A. (CDR), Campbell, J. (CAPT), *Molecular Biological Characterization of Air Samples: A Survey of Four Strategically Important Regions*, Naval Research Laboratory, NRL/MR/6110-03-8661, #20030312 219, January 31, 2003.

⁴⁰ Birmingham, W., *Description Portal Shield Trigger Algorithm*, PSTRIG.DOC, Sentel Corporation, February 1998.